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Title

Generation of serrated and wavy petals by inhibition of the activity of TCP transcription factors in *Arabidopsis*

Running title

Petal development regulated by TCP transcription factors

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TCP transcription factors regulate the activities of ASYMMETRIC LEAVES1 and miR164, as well as the auxin response, during the differentiation of leaves in *Arabidopsis*.
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Abstract

The final shape of shoot lateral organs, namely, leaves and flowers, is determined by coordinated growth after the initiation of primordia from shoot meristems in seed plants. This coordination is achieved by the complex action of many transcription factors, which include the TEOSINTE BRANCHED1, CYCLOIDEA, and PCF (TCP) family. We have recently reported that *CINCINNATA*-like (*CIN*-like) *TCP* genes act dose-dependently to regulate the flat and smooth morphology of leaves in *Arabidopsis thaliana*. In contrast, the roles of *CIN*-like *TCP* genes in flower development are poorly understood. In this report, using multiple *tcp* mutants and transgenic plants in which the activity of *CIN*-like *TCP* transcription factors is dominantly inhibited, we found that these TCPs regulate the smooth and flat morphology of petals. Based on these findings, we discuss a possible strategy to generate a fringed morphology in floricultural plants.

Key words

TEOSINTE BRANCHED1, CYCLOIDEA, and PCF (TCP); petal; SRDX;
Arabidopsis; fringe; floriculture

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TCP genes encode proteins that contain a plant-specific DNA binding domain and regulate diverse aspects of plant development.¹ Eight of the 24 *TCP* genes in the *Arabidopsis* genome are classified as *CIN*-like *TCP* genes, since these genes have similar nucleotide sequences and functions in planta to *CIN* of *Antirrhinum majus*.^{2,4} We have demonstrated that the differential activity of *CIN*-like *TCP* genes generates a graded change in leaf forms.³ Our molecular analyses have clarified that *CIN*-like *TCP* transcription factors directly regulate genes involved in the promotion of leaf differentiation, production of miRNA, and the response to the plant hormone auxin. The products of these target genes negatively and cooperatively regulate the expression of *CUP-SHAPED COTYLEDON (CUC)* genes, which regulate the formation of shoot meristem and the morphological separation of an organ from adjacent organs. Considering these observations, we have proposed that the negative regulation of *CUC* genes by *CIN*-like *TCP* transcription factors is the core process in the development of leaves. In contrast, *TCPI*, a *CYCLOIDEA*-like *TCP* gene, regulates the longitudinal elongation of leaves,⁵⁻⁷ suggesting that the individual *TCP* genes of the different subgroups have specific functions in leaf development. In addition to the clarification of the roles of *TCP* genes during leaf development,^{3,4} we present here some insights regarding roles of *CIN*-like *TCP* genes in flower development.

We found that *CIN*-like *TCP* genes regulate petal development, using various lines of *Arabidopsis tcp* mutants³ (Figures 1A to 1H). Wild-type petals had a smooth margin and flat surface (Figures 1A and 1E), whereas the quadruple and quintuple mutants *tcp3 tcp4 tcp5 tcp10* (*tcp3/4/5/10*) and *tcp3 tcp4 tcp5 tcp10 tcp13* (*tcp3/4/5/10/13*), respectively, exhibited wavy and serrated petals (Figures 1B, 1C, 1F and 1G). Moreover, ectopic expression of *miR319A* under the control of the Cauliflower Mosaic Virus (CaMV) 35S

promoter in *tcp3/4/5/10/13* (*Pro35S:miR319A tcp3/4/5/10/13*), which resulted in suppression of *TCP2* and *TCP24*^{3,8} as well as the inactivation of *TCP3/4/5/10/13* genes, induced severely wavy and serrated petals (Figures 1D and 1H). These results suggest that *CIN*-like *TCP* genes regulate the smoothness of the surface and margin of petals. In contrast, each single mutant, and the double mutants *tcp3/tcp4*, *tcp3/tcp10*, and *tcp3/tcp5*, exhibited normal flowers, while the triple mutant *tcp3/tcp4/tcp10* showed a small sign of wavy petals (data not shown). Therefore, *CIN*-like *TCP* genes may redundantly regulate flower development, as was observed in leaf development.³

We further examined the roles of *CIN*-like *TCP* genes in petal development, using the *TCP5SRDX* gene, in which *TCP5* was fused with *SRDX*, a strong transcriptional repression domain^{4,9} (Figures 1I to 1P). When we expressed *TCP5SRDX* under the control of the CaMV 35S promoter (*Pro35S:TCP5SRDX*) in *Arabidopsis* plants,⁴ we found that *Pro35S:TCP5SRDX* plants had severely serrated margins of sepals and petals (Figures 1I to 1L), in addition to wavy and serrated leaves.⁴ This abnormality was possibly caused by the dominant repression of expression of the putative target genes of *CIN*-like *TCP* transcription factors.^{4,9} Since the phenotype of *Pro35S:TCP5SRDX* petals resembled--but was more severe than--*Pro35S:TCP3SRDX* petals,⁴ we compared the abaxial epidermis of *Pro35S:TCP5SRDX* and wild type petals by scanning electron microscopy. The wild-type petals had rounded cells in the distal region and flat cells elongated according to the proximodistal axis in the proximal region (Figures 1M and 1O). In contrast, *Pro35S:TCP5SRDX* petals had flat and elongated cells in all regions examined, and did not have rounded cells in the distal region (Figures 1N and 1P). The flat cells in *Pro35S:TCP5SRDX* petals resemble those in the *cin* mutant.¹⁰ The altered regulation of cell differentiation in the distal region of *Pro35S:TCP5SRDX* petals might

interfere with the patterning of the smooth margin.

Extending our finding that inhibition of the activity of CIN-like TCP transcription factors induces a wavy and serrated margin of *Arabidopsis* petals, we introduced the *TCPSRDX* genes into several floricultural plants, and have recently generated transgenic plants with modified petals. In *Cyclamen persicum*, expression of *CpTCP1SRDX*, which is derived from an endogenous CIN-like *TCP* gene, induces ruffled petals.¹¹ Furthermore, expression of *TCP3SRDX*, even if it is derived from the *Arabidopsis* genome, induces fringed and wavy petals in *Torenia fournieri* and *Rosa x hybrida*, respectively.^{12,13} In addition, these modified petals exhibit a variation of coloring pattern. Interestingly, *TCP3SRDX* induces a specific morphology of petals with suppressed development in *Chrysanthemum morifolium*.¹² Chrysanthemum plants have a pseudanthium composed of a disk and ray florets, and the effect of *TCP3SRDX* could result in a different morphology of the chrysanthemum pseudanthium. With respect to leaf morphology, expression of the *TCPSRDX* genes induces undulation, serration, or dissection with different severity in the plants that we examined.¹¹⁻¹³ Therefore, using the *TCPSRDX* genes, we provide a simple methodology for generating leaves and petals with different margins and surfaces.

In this report, we demonstrate that CIN-like *TCP* genes regulate the smooth morphology of petals, and these genes act redundantly to regulate petal development in *Arabidopsis*. Considering the similarity in the roles of CIN-like *TCP* genes during the development of both leaves and petals,^{3,4} we postulate that the molecular basis downstream of CIN-like TCP transcription factors is conserved, in part, among several different plants. One possible conserved gene downstream of CIN-like TCP transcription factors is that involved in the separation of organs.^{3,4} Although *CUC* genes are involved in the separation of leaves as well as sepals and stamens,¹⁴ it is important to identify genes

responsible for the separation of petals.

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Figure legend

Figure 1. Flowers of wild type, *tcp* mutants, and *Pro35S:TCP5SRDX* plants.

Top views of wild-type (A), *tcp3/4/5/10* (B), *tcp3/4/5/10/13* (C), and *Pro35S:miR319A tcp3/4/5/10/13* (D) flowers. Petals of wild type (E), *tcp3/4/5/10* (F), *tcp3/4/5/10/13* (G), and *Pro35S:miR319A tcp3/4/5/10/13* (H) plants. The photos in (E) to (H) are presented in back and white for detailed visualization of the margin. Side views of wild-type (I) and *Pro35S:TCP5SRDX* flowers (J). Petals of wild type (K) and *Pro35S:TCP5SRDX* plants (L). The moderate and severe phenotypes of *Pro35S:TCP5SRDX* petals are presented at left and right, respectively, in (L). The epidermal cells at the distal region of wild-type (M) and *Pro35S:TCP5SRDX* (N) petals. The epidermal cells at the proximal region of wild-type (O) and *Pro35S:TCP5SRDX* (P) petals. Bars = 0.5 mm in (A) to (L) and 10 μ m in (M) to (P).

